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SHP1 is a cytosolic protein tyrosine phosphatase containing two SH2 domains. It is highly expressed in hematopoietic cells and at lower levels in epithelium. We introduced SHP1, both in its wild type (wt) or a catalytic inactive (dn) form, into the human breast cancer cell line MDA-MB231, where SHP1 expression is nearly lost. The overexpression of wt SHP1, but not dn SHP1, led to enhanced anchorage-independent growth, as well as enhanced tumorigenicity, without altering cellular proliferation rate. Our results suggest that such an enhancement in tumorigenicity is not caused by increased angiogenesis. The overexpression of SHP1 does not affect the magnitude of EGF signaling in the MDA-MB231 cell, but rather affect the duration of signaling. The overexpression of SHP1 in the MDA-MB231 cells led to an average 15% increase in G0/G1 phase population during cell cycle and a corresponding drop in S phase. SHP overexpression in this cell cline did not lead to any increase in Src protein tyrosine kinase activity. A study is underway to identity a potential downstream effector of SHP1, which has a molecular weight of 40 kDa with a pI between 5.1 and 6.6.

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INTRODUCTION

SHP1 is a cytosolic protein tyrosine phosphatase that contains two SH2 domains. It is highly expressed in hematopoietic cells and expressed in normal epithelium at lower levels. While SHP1 in hematopoietic cells is thought to be a negative regulator of cellular signaling by associating with and dephosphorylating various receptors and their downstream effectors after they become activated, its precise function in epithelium remains to be understood. The potential involvement of SHP1 in human tumorigenesis has been hypothesized from the findings that SHP1 can interact with, dephosphorylate, and regulate the activity of several protein tyrosine kinases (PTKs) implicated in human cancer. These PTKs include epidermal growth factor receptor (EGFR) and Src. Such speculation is also supported by the report that SHP1 is overexpressed in human ovarian cancers.

We discovered, as stated in our first annual report, that the levels of SHP1 expression and activity are altered in human breast cancer cells in comparison with normal breast epithelium. In particular, SHP1 expression is nearly lost in the human breast cancer cell line MDA-MB231. After the re-introduction of SHP1, both in wild type (wt) and enzymatically inactive (dn) forms, into the MDA-MB231 cells, we observed no changes in cellular proliferation. However, the overexpression of wt SHP1 led to increased anchorage-independent growth in the MDA-MB231 cells, as well as enhanced tumorigenicity in nude mice. SHP1 phosphatase activity is essential for such positive effects since the overexpression of dn SHP1 had no effect.

In this report, we will introduce results from additional experiments to confirm that the overexpression of wt SHP1, but not dn SHP1, leads to enhanced tumorigenicity in the MDA-MB231 cells. We will also discuss some of our new observations in an attempt to understand the molecular mechanism of such an enhancement.

RESULTS

I. The effect of wt SHP1 overexpression on tumorigenicity

cells (1 x 10^6 of each cell line, including the parental cells MDA-MB231 and the stable transfectants) were injected into the mammary fat pad of a group of five 6-8 week old female nude mice. Tumor progression was monitored twice a week and followed for up to 7-8 weeks after the injection. The transfectants expressing wt SHP1, but not dn SHP1, produced larger tumors than the parental cells and the pcDNA3 (neo) transfectants.

To rule out the potential influence of clonal variation, a similar yet not identical experiment was also conducted to study the effect of SHP1 on tumorigenicity. In this experiment, the pooled clones of the MDA-MB231 cells stably transfected with either the vector pcDNA3 (neo), wt SHP1, or dn SHP1, instead of the individually selected clones, were injected into nude mice. Again, wt SHP1 pooled clone produced large tumors, whereas the pooled neo clone and dn SHP1 clone produced smaller, similar sized tumors.

Based on the results of these experiments, we concluded that overexpression of SHP1 in the MDA-MB231 cells led to enhanced tumorigenicity and that the enzymatic activity of SHP1 was crucial for such a positive effect.

II. Angiogenesis study

We studied the levels of angiogenesis in the tumors recovered from nude mice in order to understand whether the overexpression of wt SHP1 led to more advanced angiogenesis with subsequently larger tumors. Tumors originated from the parental cells and three types of clones were sectioned and stained for CD31 (also referred to as PECAM-1, Platelet Endothelial Cell Adhesion Molecule), a surface marker for cells of endothelial origin, to illustrate the degree of vascularization, i.e. angiogenesis, of these tumors. Based on such immunohistochemical staining of numerous tumor sections, we concluded that there was no significant difference in the degree of angiogenesis among

the tumors originated from the MDA-MB231 cells and the transfectants. Therefore, we concluded that in the MDA-MB231 cells the overexpression of wt SHP1 promoted tumor growth by a mechanism or mechanisms apart from promoting angiogenesis.

III. The effect of SHP1 overexpression on the kinetics of EGFR tyrosine phosphorylation

We previously observed no significant changes in the peak level of EGFR tyrosine phosphorylation upon EGF stimulation in the MDA-MB231 cells overexpressing either wt or dn form of SHP1. In our recent experiments, we discovered that the overexpression of SHP1 in the MDA-MB231 cells slightly altered the kinetics of EGFR dephosphorylation following EGF stimulation: overexpression of wt SHP1 led to slightly accelerated EGFR dephosphorylation, whereas overexpression of dn SHP1 led to slightly delayed EGFR dephosphorylation. We therefore concluded that SHP1 had slight effect on the duration of EGF signaling and no effect on the magnitude of EGF signaling.

IV. The effect of SHP1 overexpression on cell cycle progression

We also studied potential effect of SHP1 overexpression on cell cycle progression. The MDA-MB231 cells were transiently transfected with pEFBOS-mGFP, a plasmid encoding membrane-bound green fluorescent protein (mGFP), and one of the pcDNA3 (neo), wt SHP1, or dn SHP1 at a molar ratio of 1:4. They were then fixed between 48-72 hours after the transfection. Using a flowcytometer, only those cells exhibiting green fluorescence were gated and their cell cycle status assessed. The results of several independent experiments indicated that the overexpression of wt SHP1 in the MDA-MB231 cells led to an average 15% increase in the G0/G1 phase, whereas the cell population in the S phase showed a decrease.

V. The effect of SHP1 overexpression on Src kinase activity

The report that SHP1 can dephosphorylate an inhibitory tyrosine site in Src PTK and subsequently activate Src kinase activity prompted the studies to determine if the overexpression of wt or dn SHP1 in the MDA-MB231 cells led to any changes in the kinase activity of Src. The kinase assays were conducted, following immunoprecipitation, with the MDA-MB231 cells and three types of stable transfectants. The results of these kinase assays suggested that the overexpression of SHP1, regardless of its catalytic activity, led to no increase in Src kinase activity.

VI. Identification of potential downstream effector(s) of SHP1

Since the catalytic activity of SHP1 is necessary for its effect in enhancing tumorigenicity, we deduced that such an enhancement in tumorigenicity must involve changes in the tyrosine phosphorylation status of certain protein(s), as a result of wt SHP1 overexpression. The identification of such protein(s) should provide vital information in understanding the cellular events leading to enhanced tumorigenicity in the MDA-MB231 cells upon the overexpression of wt SHP1.

Using two-dimensional electrophoresis, we have discovered one cellular protein whose tyrosine phosphorylation level is consistently higher in the MDA-MB231 transfectants where wt SHP1 is overexpressed. This protein has an apparent molecular weight of about 40 kDa, which we thus term p40, with a pI between 5.9 to 6.6. Since p40 showed increased tyrosine phosphorylation at the presence of an active tyrosine phosphatase, we concluded that there must be other protein(s) involved in the signaling pathway between SHP1 and p40. We are currently searching for the identity of p40.

APPENDIX

DEGREE OBTAINED:

Chuan Gao, who has been supported by this award since August 1997, received a Ph.D. degree in August 1999.